

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
29 March 2001 (29.03.2001)

PCT

(10) International Publication Number  
**WO 01/20985 A1**

(51) International Patent Classification<sup>7</sup>: **A01N 25/24**,  
A61K 7/46, C08L 1/10, 3/06, C11D 3/50, D06M 15/11,  
B01J 20/26, 13/04

NL-6703 GL Wageningen (NL). LENSELINK, Willem  
[NL/NL]; Molenweg 97, NL-3781 VC Voorthuizen (NL).

(21) International Application Number: PCT/NL00/00603

(74) Agent: PRINS, A., W.; Vereenigde, Nieuwe Parklaan 97,  
NL-2587 BN The Hague (NL).

(22) International Filing Date: 30 August 2000 (30.08.2000)

(25) Filing Language:

Dutch

(26) Publication Language:

English

(30) Priority Data:

1012933

30 August 1999 (30.08.1999) NL

(71) Applicant (for all designated States except US): PFW  
AROMA CHEMICALS B.V. [NL/NL]; Nijverheidsweg  
60, NL-3771 ME Barneveld (NL).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (for US only): VAN SCHIJNDEL,  
Renée, Josie, Gide [NL/NL]; Schaepmanstraat 117,  
NL-6702 AS Wageningen (NL). SIVASLIGIL, Dogan,  
Sahin [NL/NL]; Wouwermanstraat 139, NL-2525 KR  
The Hague (NL). WESTERWEELE, Eddie [NL/NL];  
Dorpsstraat 98, NL-6871 AP Renkum (NL). VAN SOEST,  
Jeroen, Johannes, Gerardus [NL/NL]; Broekemalaan 2,

Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/20985 A1

(54) Title: IMMOBILIZATION OF ACTIVE SUBSTANCES

(57) Abstract: The invention relates to a method for immobilizing an active substance, wherein a mixture is prepared of the active substance and a carrier material in a liquid phase, whereafter the liquid phase is converted to a solid phase, the carrier material being an esterified polysaccharide. The invention further relates to the use of an esterified polysaccharide for fixing or immobilizing active substances, in particular odorous substances.

**Title:   Immobilization of active substances**

This invention relates to a method for immobilizing active substances.

In the literature, different methods are known for the immobilization of active substances. The purpose of the immobilization is normally to achieve a slowed and/or controlled release of the active substance. Substances that are used as carrier material on which the active substances are immobilized vary in nature. To be mentioned by way of example are synthetic polymers and biopolymers such as starch or alginates.

International patent application 89/03674 discloses a method for preparing microspheres by suspending an active substance, such as paramagnetic particles, in a starch solution, crosslinking the starch with a phosphate, and emulsifying the starch in a hydrophobic medium before or after crosslinking.

European patent application 0 930 334 discloses a polysaccharide conjugate which is capable of binding cellulose. The conjugate is based on a polysaccharide, which is not modified, and a particle that carries a perfume. The particle is preferably a porous silica particle, into which the perfume can penetrate through diffusion.

U.S. Patent 5,667,803 relates to the use of a starch acetate as auxiliary substance in pharmaceutical compacted compositions, mainly tablets. Depending on the degree of substitution (DS) of the starch acetate, it is to be used as disintegrator, filler, binder, or agent for regulating the release of an active substance from a tablet. No mention is made of the immobilization of an active substance on a starch acetate. Accordingly, the starch acetate itself cannot, in the application of this publication, be regarded as carrier material.

International patent application 93/02712 discloses a method in which an oil-in-water emulsion of a soluble starch fraction and an organic

solvent, such as dichloromethane, is prepared, to which a dehydrating agent such as an alcohol is added. The thus obtained microspheres are fixed by retrogradation of the starch, which must therefore have a high amylose content.

5 Dutch patent application 10.06444 proposes an improvement of the above-mentioned immobilization methods. According to the method described therein, microparticles consisting of an effective substance in a starch envelope are prepared by preparing an oil-in-water emulsion of the effective substance in a hydrophobic phase and starch in water, including  
10 this emulsion in a second hydrophobic phase, and subsequently crosslinking the starch. Optionally, the second hydrophobic phase can eventually be removed. A disadvantage of this method is that with different types of effective substances it has been found that high degrees of loading of the effective substance in the microsphere are not feasible.

15 U.S. Patents 3,455,838 and 5,354,559 and the British Patent Specification all relate to the encapsulation of active substances with water-soluble or shortened starches, which are optionally substituted. The degree of substitution (DS) of the starches described is low in each case. The encapsulation in each case takes place from an emulsion or with the aid of a  
20 spray-drying technique. A disadvantage of the systems described is their water-sensitivity. When used in an aqueous medium, for instance during a washing process, the capsules will easily disintegrate, so that the active substance is released into the water at an undesired time.

Surprisingly, it has presently been found that the above-mentioned  
25 disadvantages can be obviated by using a specific carrier material for the immobilization of an active substance. The specific carrier material is an esterified polysaccharide. The invention thus relates to a method for immobilizing an active substance, wherein a mixture is prepared of the active substance and a carrier material in a liquid phase, whereafter the

liquid phase is converted to a solid phase, the carrier material being an esterified polysaccharide.

According to the invention, highly stable systems are obtained. This is understood to mean that it is accomplished that an active substance  
5 which is immobilized according to the invention is substantially not released under undesired conditions and is substantially released only under desired conditions. Thus, according to the invention, a particularly favorable slowed-release profile can be set. This is also understood to mean that through the immobilization the active substance is protected, so that  
10 the chance of breakdown of the active substance through physical or chemical influences is reduced considerably. Thus, an active substance immobilized according to the invention will have a prolonged shelf life. Further, according to the invention, it has been found possible to load a carrier material with very large amounts of active substance.

15 Further, it is a great advantage of the manner of immobilization according to the invention that it can be effected very simply. Virtually no (complex) operations need to be performed to obtain the stable system referred to.

As used in this text, the term "immobilized active substance" refers  
20 to a complex of an active substance and a carrier material.

As said, the carrier material that is used according to the invention is an esterified polysaccharide. Examples of suitable polysaccharides are starch, cellulose, alginates, pectin and combinations thereof. Preferably, the polysaccharide is starch or cellulose, starch being particularly preferred.  
25 With esterified starch, very high loading degrees are feasible. In principle, the starch can originate from any natural starch source. Suitable, among others, is starch coming from potatoes, corn, wheat, and tapioca. Preferably, granular starch is used. Optionally, the starch can be wholly or partly gelatinized.

To obtain the desired esterified polysaccharide, it is possible to start from the native polysaccharide or from a derivative thereof. Suitable derivatives in this connection are, for instance, (partially) hydrolyzed polysaccharides, oxidized polysaccharides, ionized (both cationic and anionic) and etherified polysaccharides. Incidentally, it will be clear that the reaction that is performed starting from native polysaccharide to obtain any one of the derivatives mentioned can also be performed with the polysaccharide already esterified.

The esterified polysaccharide is preferably biodegradable. In the context of the invention, a biodegradable material is understood to mean a material that has the property of being broken down within a relatively short time into substances that are preferably soluble in water and non-toxic. The breakdown can take place *inter alia* through hydrolytic cleavage, under the influence of light, air, water and/or microorganisms occurring in nature.

The esterification proper can be carried out in any known manner. The polysaccharide can, for instance, be subjected to a reaction with an acid anhydride, which provides the desired ester group, in aqueous, slightly alkaline medium. Examples of suitable esterification reactions are to be found in R.L. Whistler, E.F. Paschall, "Starch Chemistry and Technology", vol. 1 & 2 (1965), Academic Press Inc. The esterification is preferably carried out so as to yield a degree of substitution (DS) at which the esterified polysaccharide is not soluble or poorly soluble in water. In view of this criterion, the desired degree of substitution depends on the nature of the ester group. When the ester group is relatively non-polar, the value of the degree of substitution can be between 0.05 and a DS corresponding to a complete substitution, and preferably, in particular, between 0.1 and 2.7. In the case of a less non-polar ester group, such as an acetate group, the degree of substitution is preferably slightly higher, viz. between 0.3 and 3, preferably between 0.3 and 2.7. It has been found that the release rate of

hydrophilic active substances is higher at a relatively high DS, while the release rate of hydrophobic active substances is higher at a relatively low DS.

Suitable ester groups that can be introduced are *inter alia* acetate groups, propionate groups, butyrate groups, alkyl succinate groups, in which the alkyl group contains from 1 to 16 carbon atoms, benzoate groups, and ester groups which are derived from carboxylic acids having from 1 to 18 carbon atoms, such as saturated and monounsaturated or polyunsaturated fatty acids. Preferably, an acetate ester of a polysaccharide is used, because an active substance can be immobilized with it in a particularly stable manner.

The active substance which is immobilized according to the invention can be selected from *inter alia* medicines (for instance hormones, antiinflammatory agents, insulin, chemotherapeutics, antibiotics, vaccines and the like), plant protection agents (such as atachloride), paramagnetic substances, catalysts, organic reactants, pheromones, lures, cosmetic actives, washing active substances, disinfectants, fabric conditioning actives, hair conditioning actives, colorants, fragrance, flavor, and nutrients (for instance vitamins, fats, proteins, peptides, etc.). Naturally, combinations of the active substances mentioned can be immobilized. Preferably, an active substance is used which is soluble or dispersible in a hydrophobic phase.

In a preferred embodiment of the invention, the active substance is an odorous substance or fragrance. In the context of the invention, fragrance is understood to mean a compound which releases a particular desired odor. Fragrance is also understood to mean a mixture of compounds which is so composed that the odors of the different components of the mixture jointly release a pleasant or desired odor. Examples of compounds that can be used, alone or in combination, as fragrance are natural oils, vegetable and animal extracts, synthetic oils, alcohols, aldehydes, ketones,

esters, lactones, ethers, hydrocarbons, nitriles and other classes of chemical compounds. Fragrance can be used to impart to the environment, or other compounds or compositions, a modified, different or enhanced odor.

By immobilizing a fragrance in accordance with the invention, a highly favorable release pattern of the desired odor is accomplished. Additionally, by virtue of the high loading degrees that are feasible, a more intense or longer release pattern than before can be accomplished. Moreover, it has been found that the shelf life of fragrances is greatly extended by immobilizing them according to the invention.

10 In order to immobilize the active substance on the carrier material, a homogeneous mixture of the active substance and the carrier material in a liquid phase is formed. This can be done in different ways.

Depending on the nature of the carrier material and the active substance, by heating a mixture of the two, a liquid phase can be formed. In the liquid phase, a very homogeneous mixture can be obtained, for instance by stirring. Subsequently, by cooling, a solid phase can be formed, in which the active substance is immobilized on the carrier material.

It is also possible to form a solution or dispersion of the carrier material and the active substance in a suitable solvent, so that the liquid phase is formed by the solvent. By evaporating the solvent, the solid phase can be obtained, in which the active substance is immobilized on the carrier material. Suitable solvents can be obtained depending on the nature of the carrier material and the active substance. Preferably, the solvent has a relatively low boiling point. Examples of solvents that can be used are acetone, diethyl ether, dichloromethane, ethanol, methanol and isopropanol.

Incidentally, it is also possible to combine the two possibilities and to prepare a melt of the carrier material and the active substance in the presence of a small amount of a solvent, such as the solvents mentioned earlier.

In a preferred embodiment, small particles, such as microspheres, of immobilized active substance can be prepared by making use of the solvent evaporation method, known per se. It involves the preparation of an emulsion of the above-mentioned liquid phase. The additional liquid that is  
5 needed for that purpose is preferably water, so that an oil-in-water emulsion is obtained. If desired, a suitable emulsifier, for instance polyethylene glycol, can be used. This emulsion is subsequently dried, whereby the intended particles are formed. These can be isolated by, for instance, centrifugation.

10 In addition, it is possible to make use of the so-called double-emulsion technology as described in Dutch patent application 10.06444. This involves encapsulation of an active substance by preparing an oil-in-water emulsion of the active substance in a first hydrophobic phase and a solution or suspension of the carrier material in an aqueous starch dispersion or  
15 solution, which oil-in-water emulsion is subsequently incorporated in a second hydrophobic phase. When this technology is used in the context of the present invention, the active substance is included together with the carrier material in the first hydrophobic phase. As a result, it is possible to set the viscosity of this first hydrophobic phase, which makes the  
20 encapsulation simpler. Suitable materials for use as first and second hydrophobic phase can be simply found by one skilled in the art on the basis of the Dutch patent application mentioned in combination with his own expert knowledge.

25 According to another embodiment, a gaseous active substance is immobilized on the above-described carrier material. In order to achieve this, the carrier material can be employed in a liquid phase. This phase can be obtained by heating the carrier material or by dissolving or dispersing it in a suitable solvent. If desired, after immobilization of the gaseous active substance, the liquid phase can be converted to a solid phase by cooling or



removing the solvent. It is also possible to immobilize the gaseous active substance by contacting it with the carrier material in solid form.

According to the invention, it has been found possible to absorb active substances, in particular odorous substances. It has been found that  
5 the present carrier material is highly suitable for capturing odorous substances. Thus, for instance, fabric that has an unpleasant smell can be treated with the carrier material, as described above, so that the odorous substance spreading the undesired odor is immobilized on the carrier material. What is thus achieved is that the odor is reduced, at least is less  
10 perceptible. The odorous substance that is captured can be in the solid, liquid or gaseous phase. It has been found that when the odorous substance is in the gaseous form, it can be captured by the carrier material in solid form. In addition, the carrier material can be used in a liquid phase to absorb the odorous substance. After optional drying or cooling, the solid,  
15 immobilized active substance can be simply removed. Thus, the invention also relates to the use of an esterified polysaccharide for fixing or immobilizing an active substance, the active substance being preferably an odorous substance.

This embodiment of the invention can be suitably used for removing  
20 numerous unpleasant odors, for instance caused by body fluids, (armpit) deodorants, personal care products, such as (incontinence) diapers, sanitary napkins, panty-liners, tissues, (paper) napkins or towels, and toilet paper, air fresheners, space deodorants, cat litter. In a preferred embodiment, a product, such as (incontinence) diapers, sanitary napkins, panty-liners,  
25 tissues, paper napkins or towels, toilet paper or cat litter, can be provided with the carrier material according to the invention, so that in use the products spread less unpleasant odors.

The degree of loading that can be achieved according to the invention is partly dependent on the nature of the carrier material, the active  
30 substance and the application. Mostly, loading will be between 0.1 and 99%

by weight, in particular between 1 and 50% by weight, based on the total weight of dry matter of the carrier material.

If desired, the solid, immobilized active substance, which may or may not be modified as described above, can be processed to form a powder, for instance by grinding. The particle size of the powder can be selected depending on the intended application of the immobilized active substance. It is also possible to use the immobilized active substance in larger forms. Such forms can be obtained by the use of conventional polymer shaping techniques such as extrusion, including film and foil extrusion, injection molding, pressing or vacuum drawing.

In order to influence the processability and applicability of the immobilized active substance, a chemical or physical modification can be performed on the surface of the carrier material. Examples of suitable modifications are partial hydrolysis of the carrier material, crosslinking of the carrier material and ionization of the carrier material, and combinations thereof. It will be clear to one skilled in the art that a variety of modifications known per se are eligible under given circumstances. Preferably, only a part of the carrier material, preferably less than 5% by weight, more preferably less than 1% by weight, of the total amount of carrier material is modified.

Partial hydrolysis of the carrier material leads to the immobilized active substance acquiring a more hydrophilic character, which may be desirable when applications in aqueous medium are contemplated. The hydrolysis can be carried out, for instance, by suspending particles of the carrier material in water. Optionally, a small amount (0.1 - 2% by weight, based on the weight of the particles) of surfactant, such as sodium dodecylsulfate or an alkyl (poly)glucoside can be added. If desired, the hydrolysis can be accelerated by changing the pH of the suspension or raising the temperature. Preferably, work is done at a pH between 8 and 13 and a temperature between 20 and 40°C.

Crosslinking of the surface of the particles can be carried out to make the immobilized active substance less soluble in water. This may be desired after a partial hydrolysis or other modification has been carried out. When crosslinking is used in combination with a partial hydrolysis, an  
5 immobilized active substance is obtained which does not dissolve in water but can be very well incorporated in water, for instance in the form of a suspension. In addition, crosslinking leads to the active substance being retained particularly well by the carrier material. Crosslinking can be carried out by a reaction with a suitable crosslinking agent, such as  
10 trisodium phosphate or epichlorohydrin. To that end, for instance, a suspension is prepared of the particles in water, optionally in the presence of a small amount (0.1 - 2% by weight, based on the weight of the particles) of surfactant, such as sodium dodecylsulfate or an alkyl (poly)glucoside, to which the crosslinking agent is added in an amount of 0.5-3% by weight,  
15 based on the weight of the particles. Preferably, a small amount (0.5 - 5% by weight, based on the weight of the particles) of a base, for instance NaOH or KOH, is added. Other examples of possible crosslinking reactions are described in R.L. Whistler, E.F. Paschall, "Starch Chemistry and  
Technology", vol. 1 & 2 (1965), Academic Press Inc.

20 By providing positively or negatively charged groups, the interaction of the immobilized active substance with the environment can be set. What can thus be achieved is, for instance, that the immobilized active substance adheres well to other materials, such as fabric. In addition, the dispersibility of the immobilized active substance can be positively  
25 influenced. The provision of positively or negatively charged groups at the surface can in principle be carried out in any known manner for introducing charged groups into a polysaccharide material. Examples of suitable methods are described inter alia in R.L. Whistler, E.F. Paschall, "Starch Chemistry and Technology", vol. 1 & 2 (1965), Academic Press Inc.

Quaternary ammonium groups and carboxyl or phosphate groups are preferred.

The active substance can be released to a target environment by chemical, physical or enzymatic influences. Normally, these influences will (partially) break down or modify the carrier material, such that the active substance is liberated. The active substance can, for instance, be released into the digestive tract under the influence of the prevailing conditions in the various organs (pH, enzymes). Optionally, the sensitivity of carrier material can be adapted by starting from a different esterified polysaccharide derivative. In laundering agents, the release can be promoted by temperature increase or, again, by pH or enzyme influences. On or in cultivated soil or potting soil, the release can be accomplished by hydrolysis or action of salts. In reaction mixtures, the release can also be accomplished by the influence of, for instance, electric current or pH adjustment.

The immobilized active substance can be used in various applications. Examples include washing agents, fabric softeners, cleaning agents (such as cleaners, detergents, disinfectants, washing-up agents, dish-washing agents, rinsing agents, bleaching agents, and toilet cleaners), fabric conditioners, fabric sprays, ironing aids, tumble dryer additions, optical whiteners, odor masking agents, personal care products, fertilizers, foods, flavors, pharmaceutical agents, tissues, cosmetics (such as perfumes, colognes, bath and shower products, shampoos, hair conditioning products, skin care products, sun screens, creams, lotions, aerosols, and soaps), soil improvers, plant protection agents (against fungi, bacteria, insects, mites, nematodes and the like), covering layers or coatings, paints, inks, organic reactants (hydrogen peroxide), catalysis, and diagnostics.

The invention will now be further elucidated in and by the following examples.

**Exempl 1** (immobilization of a fragrance on a carrier material)

- 5 A. Paselli acetate (10 g) is dissolved in a mixture of fragrance (5 g) and acetone (5 g), optionally with heating (50°C). Next, the volatile solvent acetone is removed by heating the material at 80°C. After cooling, a hard brittle material having a fragrance loading of about 33% by weight is obtained. The material is ground, optionally cryogenically, to form a powder.
- 10 B. Paselli acetate (10 g) is dissolved in a mixture of fragrance (5 g) and acetone (5 g), optionally with heating (50°C). Next, a thin film is formed of Paselli acetate/fragrance by pouring the solution onto glass and allowing the volatile solvent acetone to evaporate.
- 15 C. Paselli acetate (10 g) is dissolved or suspended in fragrance (5 g) and heated in a closed reactor at 105°C for 2 hours. After cooling, a hard brittle material having a fragrance loading of about 33% is obtained. The material is ground, optionally cryogenically, to form a powder.
- 20 D. Paselli acetate powder (500 mg) is brought into a saturated atmosphere of fragrance. Depending on the absorption time and the temperature, the loading can be set, see also Fig. 4. The material is ground, optionally cryogenically, to form a powder.
- 25 E. Cellulose acetate (10 g) is dissolved in a mixture of fragrance (5 g) and acetone (5 g), optionally with heating (50°C). Next, the volatile solvent acetone is removed by heating the material at 80°C. After cooling, a hard brittle material having a fragrance loading of about 33% by weight is obtained. The material is ground, optionally cryogenically, to form a powder.

**Example 2** (chemical modifications on surface of the immobilized active substance)

**Example 3 (release behavior of fragrances)**

- 5 A. Figure 1 gives the release profiles of a yes- (a) and no- (b) immobilized fragrance mixture (ACB 56SE) applied to fabric. The figure clearly shows differing release behavior. The fragrance mixture is immobilized on a carrier material consisting of Paselli acetate having a DS of 3.
- 10 B. Figure 2 gives the release profiles of three immobilized fragrances, viz., Linalool (a), Tilianol Super (b) and Hydroxycitronellal (c). The figure clearly shows that the fragrances exhibit a mutually different release behavior. The fragrances are immobilized on a carrier material consisting of Paselli acetate having a DS of 3.
- 15 C. Figure 3 gives the release profiles of Jasmacyclene, immobilized on different carrier materials consisting of Paselli acetate with a DS of 1.0 (a), 1.7 (b) and 3 (c). The figure clearly shows that the active substance exhibits a lower release rate at a higher degree of substitution.

**Example 4 (absorption behavior of carrier material)**

- 20 Figure 4 gives the absorption behavior at room temperature for the fragrance Frutalone on a powdered Paselli acetate carrier material with a DS of 3. The amount of carrier material (0.1 gram (a) or 0.5 gram (b)) that is present in the saturated fragrance vapor has an influence on the absorption rate.

25

**Example 5 (adherence of immobilized fragrance to fabric during washing)**

- Paselli acetate/Tonalid powder (DS = 3; loading = 33%) is suspended in water in the presence of fabric (cotton). Washing is done at pH 10.4; T =  
30 60°C for 1 hour. Thereupon, the fabric is rinsed with water and dried. The

fragrance present on the fabric is extracted by means of dichloroethane and analyzed by means of gas chromatography. Figure 5 shows the adhesion, i.e. the percentage of the total fragrance that has adhered to the fabric during washing, for a few different modifications and a control experiment with  
5 normal, i.e. non-immobilized, Tonalid. The figure shows that the adherence of the fragrance increases due to the above-described immobilization and modifications.

In Figure 5 there are shown, from left to right: *blank* (= control, i.e. non-immobilized Tonalid); *neutral* (= immobilized Tonalid); *anionic* (=   
10 immobilized Tonalid, with anionized surface); *cationic* (= immobilized Tonalid, with cationized surface).

#### Example 6 (solvent evaporation method)

15 Two grams of acetylated starch (DS = 3) were dissolved, together with 1 gram of the fragrance frutalone, in 10 ml dichloromethane, analogously to the procedure of Example 1. The mixture obtained was emulsified in 200 ml water, utilizing 3% by weight of polyethylene glycol (Mw = 1,000) as emulsifier. An ultrasonic probe was used (50 output; 2 min.). Solvent evaporation was  
20 subsequently carried out while stirring (top-stirrer; 500 rpm) for 2 hours at room temperature and ambient pressure. The thus obtained microspheres were collected by centrifugation (27,000 g; 15 min.). Thereafter the particles were dried in air at room temperature and an air humidity of 30% RH. Measurement with GC showed the loading to be 28 wt. %.



solvent, such as dichloromethane, is prepared, to which a dehydrating agent such as an alcohol is added. The thus obtained microspheres are fixed by retrogradation of the starch, which must therefore have a high amylose content.

5 Dutch patent application 10.06444 proposes an improvement of the above-mentioned immobilization methods. According to the method described therein, microparticles consisting of an effective substance in a starch envelope are prepared by preparing an oil-in-water emulsion of the effective substance in a hydrophobic phase and starch in water, including  
10 this emulsion in a second hydrophobic phase, and subsequently crosslinking the starch. Optionally, the second hydrophobic phase can eventually be removed. A disadvantage of this method is that with different types of effective substances it has been found that high degrees of loading of the effective substance in the microsphere are not feasible.

15 U.S. Patents 3,455,838 and 5,354,559 and the British Patent Specification all relate to the encapsulation of active substances with water-soluble or shortened starches, which are optionally substituted. The degree of substitution (DS) of the starches described is low in each case. The encapsulation in each case takes place from an emulsion or with the aid of a  
20 spray-drying technique. A disadvantage of the systems described is their water-sensitivity. When used in an aqueous medium, for instance during a washing process, the capsules will easily disintegrate, so that the active substance is released into the water at an undesired time.

Surprisingly, it has presently been found that the above-mentioned  
25 disadvantages can be obviated by using a specific carrier material for the immobilization of an active substance. The specific carrier material is an esterified polysaccharide. The invention thus relates to a method for immobilizing an active substance, wherein a mixture is prepared of the active substance and a carrier material in a liquid phase, whereafter the

liquid phase is converted to a solid phase, the carrier material being an esterified polysaccharide.

According to the invention, highly stable systems are obtained. This is understood to mean that it is accomplished that an active substance which is immobilized according to the invention is substantially not released under undesired conditions and is substantially released only under desired conditions. Thus, according to the invention, a particularly favorable slowed-release profile can be set. This is also understood to mean that through the immobilization the active substance is protected, so that the chance of breakdown of the active substance through physical or chemical influences is reduced considerably. Thus, an active substance immobilized according to the invention will have a prolonged shelf life. Further, according to the invention, it has been found possible to load a carrier material with very large amounts of active substance.

Further, it is a great advantage of the manner of immobilization according to the invention that it can be effected very simply. Virtually no (complex) operations need to be performed to obtain the stable system referred to.

As used in this text, the term "immobilized active substance" refers to a complex of an active substance and a carrier material.

As said, the carrier material that is used according to the invention is an esterified polysaccharide. Examples of suitable polysaccharides are starch, cellulose, alginates, pectin and combinations thereof. Preferably, the polysaccharide is starch or cellulose, starch being particularly preferred. With esterified starch, very high loading degrees are feasible. In principle, the starch can originate from any natural starch source. Suitable, among others, is starch coming from potatoes, corn, wheat, and tapioca. Preferably, granular starch is used. Optionally, the starch can be wholly or partly gelatinized.

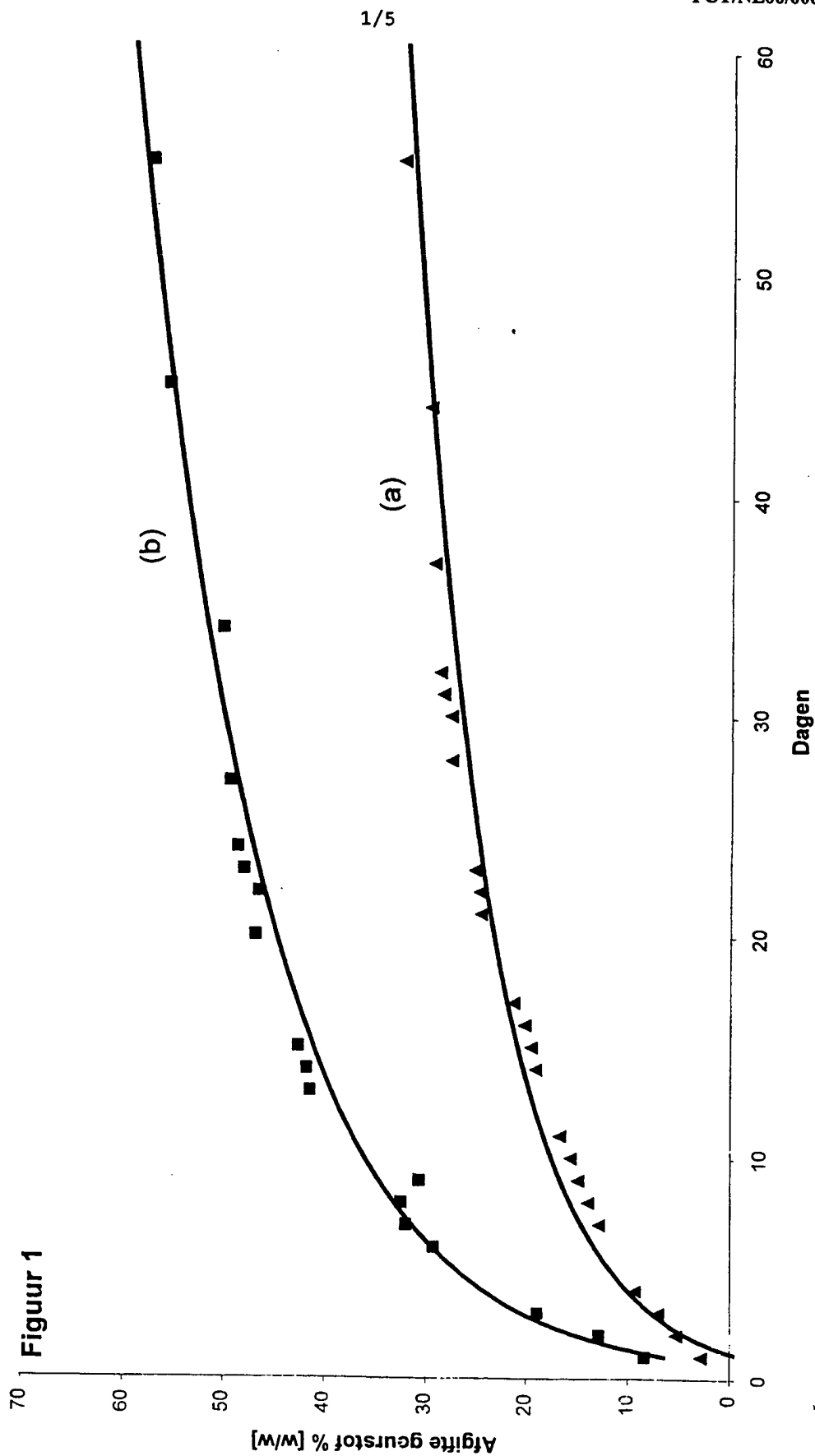
## CLAIMS

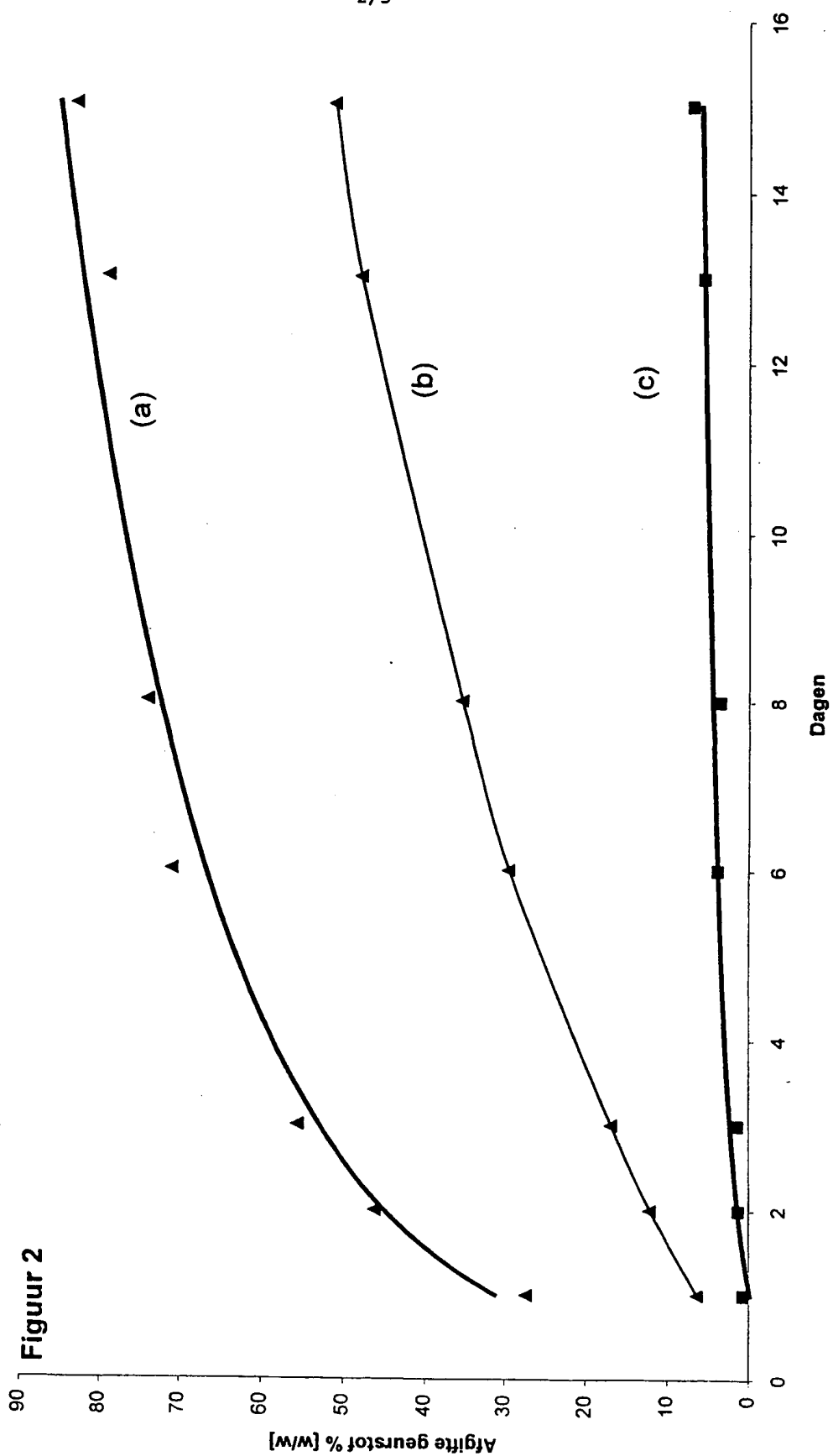
1. A method for immobilizing an active substance, wherein a mixture is prepared of the active substance and a carrier material in a liquid phase, whereafter the liquid phase is converted to a solid phase, the carrier material being an esterified polysaccharide.
- 5 2. A method according to claim 1, wherein the liquid phase is obtained by mixing and heating the active substance and the carrier material until a homogeneous liquid mixture is obtained, and wherein the solid phase is obtained by cooling.
3. A method according to claim 1, wherein the liquid phase is obtained  
10 by dissolving or dispersing the active substance and the carrier material in a solvent, and wherein the solid phase is obtained by evaporating the solvent.
4. A method according to claim 3, wherein the solvent is selected from the group of acetone, dichloromethane, diethyl ether, ethanol, methanol and  
15 isopropanol.
5. A method according to claims 1-4, wherein the liquid phase is emulsified in a liquid and wherein small particles are formed of the immobilized active substance by evaporating the liquid.
6. A method according to claim 5, wherein the liquid is water.
- 20 7. A method according to claim 1, wherein the liquid phase is a double emulsion, which is formed by preparing an oil-in-water emulsion of the active substance in a first hydrophobic phase and a solution or suspension of the carrier material in an aqueous starch solution or starch dispersion and including this oil-in-water emulsion in a second hydrophobic phase, and  
25 wherein the solid phase is formed by crosslinking the starch and removing the second hydrophobic phase.
8. A method for immobilizing an active substance, wherein the active substance is contacted in gaseous form with a carrier material in solid

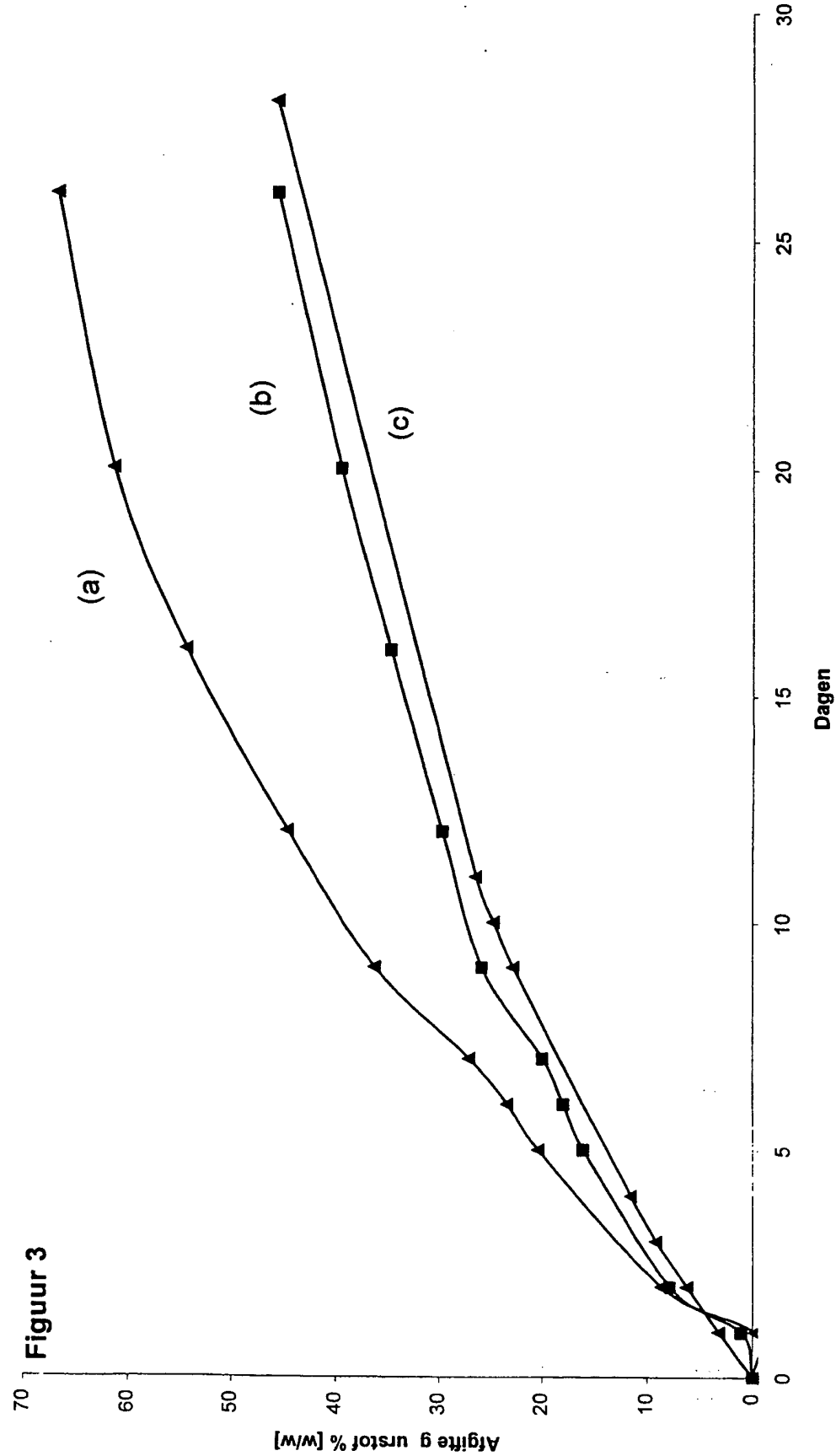
phase or a liquid phase, the carrier material being an esterified polysaccharide.

9. A method according to any one of the preceding claims, wherein the esterified polysaccharide is an esterified starch, cellulose, alginate, pectin,  
5 or a derivative thereof.
10. A method according to any one of the preceding claims, wherein the polysaccharide is esterified with an acetate group, a propionate group, a butyrate group, an alkyl succinate group, wherein the alkyl group contains  
10 from 1 to 16 carbon atoms, a benzoate group, or an ester group which is derived from a carboxylic acid having 1 to 18 carbon atoms.
11. A method according to any one of the preceding claims, wherein the polysaccharide has a degree of substitution (DS) between 0.05 and a DS corresponding to a virtually complete substitution.
12. A method according to any one of the preceding claims, wherein the  
15 active substance is selected from the group of medicines, plant protection agents, paramagnetic substances, catalysts, organic reactants, cosmetic active substances, colorants, fragrances, flavors, and nutrients.
13. A method according to any one of the preceding claims, wherein the immobilized active substance is formed into a powder.
- 20 14. A method according to any one of the preceding claims, wherein the immobilized active substance is processed by the use of polymer shaping techniques, such as extrusion, injection molding, pressing or vacuum drawing.
15. A method according to any one of the preceding claims, wherein a  
25 physical or chemical modification is performed on the surface of the immobilized active substance.
16. An immobilized active substance obtainable by a method according to any one of the preceding claims.
17. Use of an immobilized active substance according to claim 16 in a  
30 detergent, fabric softener, cleaning agent, soap, shampoo, fabric conditioner,

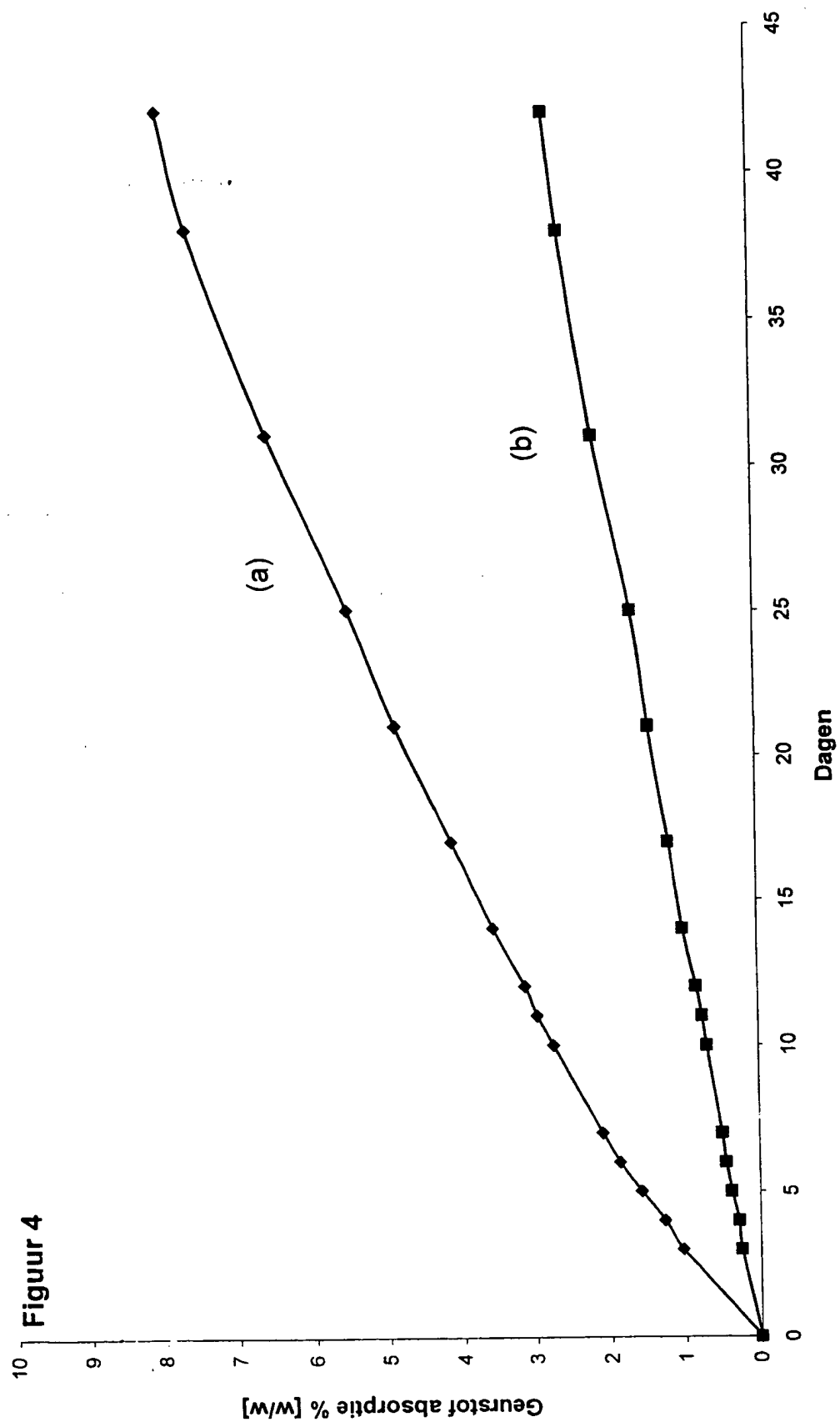
- 5 fabric spray, ironing aid, tumble dryer addition, optical whitener, odor  
masking agent, personal care product, fertilizer, food, flavor,  
pharmaceutical, tissue, cosmetics, soil improvers, plant protection agents,  
covering layer or coating, paint, ink, in organic synthesis, diagnostics or  
agriculture.
18. Use of an esterified polysaccharide for fixing or immobilizing an  
active substance.
19. Use according to claim 18, wherein the active substance is an odorous  
substance.
- 10 20. Use according to claim 19 for reducing an odor.

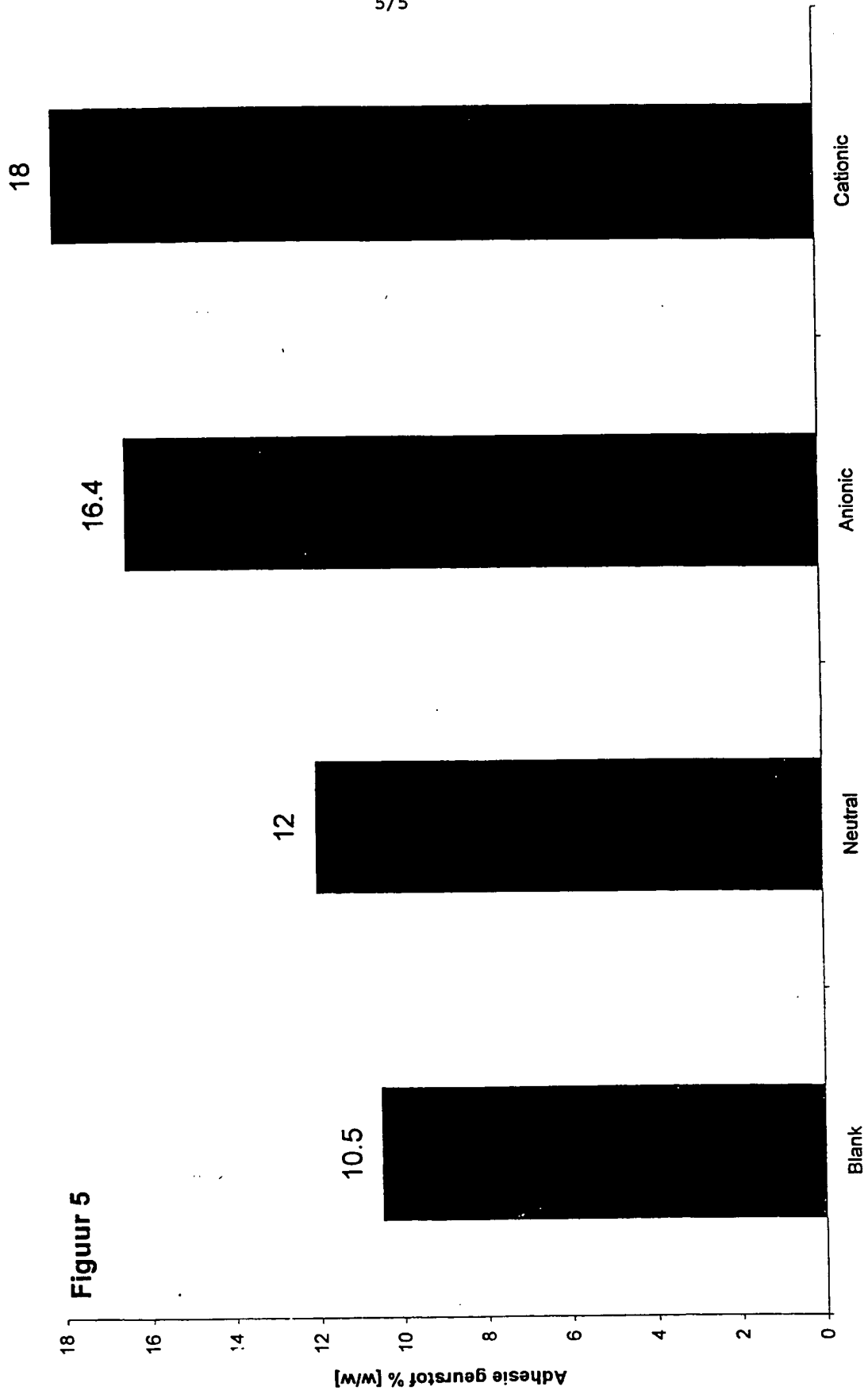












# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/NL 00/00603

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A01N25/24 A61K7/46 C08L1/10 C08L3/06 C11D3/50  
D06M15/11 B01J20/26 B01J13/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 512 693 A (DELTA BIOTECHNOLOGY LTD) 11 November 1992 (1992-11-11) page 3, line 51 -page 4, line 9 ---	1-20
X	US 4 230 687 A (SAIR LOUIS ET AL) 28 October 1980 (1980-10-28) the whole document ---	1-20
X	WO 92 11083 A (REDDING BRUCE K JR) 9 July 1992 (1992-07-09) page 11, line 7 -page 12, line 15; table 2 --- -/--	1-20

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

19 December 2000

Date of mailing of the international search report

27/12/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Reedijk, A

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/NL 00/00603

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 81, no. 22, 2 December 1974 (1974-12-02) Columbus, Ohio, US; abstract no. 140875m, page 289; XP002137793 abstract & JP 49 062623 A (FUJI PHOTO FILM) 18 June 1974 (1974-06-18) ---	1-7,9-16
A	EP 0 691 398 A (UNILEVER NV ;UNILEVER PLC (GB)) 10 January 1996 (1996-01-10) page 5, line 1 - line 27 -----	1-20

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 00/00603

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0512693 A	11-11-1992	AT 194082 T AU 655016 B AU 1589192 A AU 691196 B AU 7448394 A CA 2083260 A CN 1066977 A DE 69231195 D EP 0533886 A EP 0681843 A EP 0972526 A FI 925600 A WO 9218164 A GB 2260745 A, B HK 1006538 A HU 62805 A IL 101564 A JP 11128232 A JP 2865866 B KR 129861 B MX 9201694 A NZ 242328 A RU 2109521 C US 5993805 A US 6022525 A US 5518709 A ZA 9202636 A	15-07-2000 01-12-1994 17-11-1992 14-05-1998 22-12-1994 11-10-1992 16-12-1992 03-08-2000 31-03-1993 15-11-1995 19-01-2000 09-12-1992 29-10-1992 28-04-1993 05-03-1999 28-06-1993 20-06-1999 18-05-1999 08-03-1999 09-04-1998 01-02-1993 22-12-1994 27-04-1998 30-11-1999 08-02-2000 21-05-1996 30-12-1992
US 4230687 A	28-10-1980	NONE	
WO 9211083 A	09-07-1992	US 4978483 A AU 2551088 A AU 620571 B BR 8807221 A DK 259189 A EP 0336949 A FI 892581 A GB 2222982 A, B JP 3501940 T KR 9105190 B NO 892149 A WO 8902814 A US 5271881 A CN 1039540 A ES 2013874 A GR 89100195 A IL 89526 A MX 166283 B PT 90081 A	18-12-1990 18-04-1989 20-02-1992 17-10-1989 27-07-1989 18-10-1989 26-05-1989 28-03-1990 09-05-1991 23-07-1991 21-07-1989 06-04-1989 21-12-1993 14-02-1990 01-06-1990 31-01-1990 01-12-1992 28-12-1992 10-11-1989
JP 49062623 3 A		NONE	
EP 0691398 A	10-01-1996	CA 2153360 A	09-01-1996